Comparative effects of hypertonic solutions and pentoxifylline in sepsis

Eurasian Clinical and Analytical Medicine Original Research

Sepsis

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Abstract

Aim: Evaluation of the effects of hypertonic solutions and pentoxifylline treatments on the sepsis model.
Material and Methods: Thirty-two Sprague-Dawley rats were used. Experimental sepsis was induced by cecal ligation and puncture in all rats. At the end of the septic shock period, animals were divided into four groups, including eight rats per group, according to the treatment received as follows: sham (cannulation only, sepsis and no resuscitation), HS (3% NaCl 10 ml/kg/hr) and PTX (6 mg/kg/hr) and HS+PTX. Twenty-four hours of resuscitation after surgery, the rats were relaparotomized. Blood obtained from the heart for hematologic parameters and tissue samples (liver, lung and ileum) were collected for histopathologic analysis. The body temperature of the animals was obtained throughout the end of the experiment. Mortality rates in all groups were evaluated and compared.
Results: To assess the effects of hypertonic solutions and pentoxifylline on treating sepsis, the leukocyte, platelets and PMN values were compared. There was a significant difference between the HS Group, and PTX Group ($p<0,05$). However, there was no difference between the control Group (C) and HS+PTX Group ($p>0.05$). When fever values were compared, there was a meaningful difference between the control Group (C) and HS+PTX Group. Histopathological findings of liver, ileum and lung were compared and there was no difference between the HS Group and the PTX Group ($p>0.05$); but there was a significant difference between the control Group (C) and HS+PTX Group ($p<0,05$). The mortality rate of HS +PTX was evaluated within 24 hours, and rates of 25%, 37%, 50% and 25% were determined.
Discussion: We found Hypertonic solutions and pentoxifylline useful in the treatment of sepsis. According to the hematologic and histopathologic assessments and the evaluation of the mortality rates, early co-administration of hypertonic solutions and pentoxifylline reduces the incidence of mortality and morbidity due to septic qlinique. It is a preventive and protective method for reducing the response. Although the experimental data are positive and the rationale for the use of hypertonic solutions in the care of patients in sepsis is reasonable, many clinical studies need the safety and efficacy of sepsis treatment.

Keywords

Sepsis, Hypertonic, Pentoxifylline

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Introduction

Using hypertonic solutions advantages in the treatment of sepsis is known in the recent years compared with other crystalloid solutions. Hypertonic solutions (HS) seem to improve hemodynamics, increase organ perfusion more, and improve hypoxia more in sepsis. In addition, hypertonic solutions limit neutrophil activation, minimizing organ damage. This organ damage is mostly observed in the liver, small intestine and lung. The basic mechanism is thought to originate from the small intestine and meso. In the last decade, resuscitation with hypertonic solutions (7,5 NaCL, 4 ml/kg) in the early stages of sepsis has been found to limit the systemic immune response and inflammatory response [1].

Pentoxifylline (Ptx)(1-(5-oxohexyl)-3,7-dimethylxanthine), a methylxanthine derivation, a non-specific phosphodiesterase inhibitor, has been reported to be effective with fluid resuscitation in sepsis. Tumor necrosis factor (TNF-α) that Ptx reduces neutrophil activation known to reduce production. It has been shown that Ptx improves liver functions, modulates TNF-α and interlocking -6, improves cardiac output, increases tissue oxygenation, and increases intestinal and hepatic blood flow [2] This therapeutic effect is due to tissue perfusion, which prevents endothelial defects by correcting endothelial dysfunction and hypoxia [3-5].

Sepsis is a major public health issue responsible for approximately six million deaths per year worldwide [6]. International clinical practice guidelines and the Centers for Medicare and Medicaid Services (CMS) recommend promptly identifying sepsis and treatment with broad-spectrum antibiotic agents and intravenous fluids. These recommendations are supported by preclinical and observational studies suggesting that early treatment with antibiotics and intravenous fluids could reduce the number of avoidable deaths. However, considerable controversy exists about how rapidly sepsis must be treated [7].

The pathogenesis of sepsis is complex and involves multiple aspects of the interaction between the infecting microorganisms and the host. The recognition of pathogens and the resulting cellular activation are fundamental for infection control. Paradoxically, the host inflammatory response is also the substrate for the pathophysiological changes in sepsis [8,9]

The release of inflammatory mediators by innate immune cells upon pathogen recognition, such as tumor necrosis factor (TNF)-a, interleukin (IL)-6, and IL-1, and their effects on endothelial cells, resulting in the activation of coagulation, vasodilation, endothelial leakage, rolling and extravasation of neutrophils and inflammatory mediators to the extravascular space underscores the pathophysiology of organ dysfunctions and hypotension during sepsis [10].

Material and Methods

This study was approved by the Research Ethics Committee Of School Eskişehir Osmangazi Medicine-Unıversity of (process number/47). The experiments were performed in adherence with the international guidelines for the care and use of laboratory animals at the Laboratory Of Medicine Surgery (TICAM). Thirty-two Sprague –Dawley rats weighing between 275+-25gr were used. Anesthesia was induced with an intramuscular injection of ketamine (4 mg/100 ml) associated with xylazine (2 mg/100gr) in the same syringe. The rats were anesthetized and a small (2cm) midabdominal incision was made and the caecum and the terminal ileum were exposed. Intestinal obstruction was created by the ligation of the caecum and perforated multiple times by 22 number needle. Animals were divided into four groups according to the treatment received as follows: sham (cannulation only, sepsis and no resuscitation), HS (3% NaCl 10 ml/kg/hr), PTX (6 mg/kg/hr) and HS+PTX. The abdomens of rats were closed with nylon 3.0 sutures and the animals were maintained in individual parts for 24 h with free access to tap water.

The rats were again anesthetized with ketamine 4 mg/100 ml and 2 mg/1oo gr xylazine and relaparotomized twenty–four hours after the intestinal ischemia and obstruction, perforation. Blood obtained from the heart for hematologic parameters and tissue samples (liver, lung and ileum) were collected for histopathologic analysis. The body temperature and heart rate of animals were obtained throughout the end of the experiment.

To diagnose SIRS (Systemic inflammatory response syndrome), the presence of at least two of the following signs in the patient is sufficient.

1. The presence of hypo- or hyperthermia (Fever> 38° or< 36°).
2. Tachycardia: Pulse> 90/min.
3. Follow-up Time: Number of Breaths> 20/min. Or PaCO2<32 mmHg
4. Number of Leukocytes: > 12.000/mm3.

In our model, there were three signs of SIRS (fever, leukocytosis and tachycardia). If the SIRS is caused by an infection, it is called sepsis. In our experimental study, infection is caused by cecal ligation and perforation, so we obtained a sepsis model. Mortality rates of all groups were evaluated and compared.

Statistical Studies

All the data were obtained in this study from Eskişehir Osmangazi University Faculty of Medicine. The data were evaluated in the Department of Biostatistics. Variance analysis for hematological data, t-test and Kruskal-Wallis test were used for histopathological data.

Results

In our study, thrombocytosis was detected in all groups with sepsis. When the groups were compared with each other concerning platelet values, there was a significant difference between the thrombocyte values of the one group and the other three groups. Between the group using the hypertonic solution and the group using pentoxifylline, there was no significant difference (p>0.05). In the combination of hypertonic solution and pentoxifylline, a significant difference was found according to the group in which hypertonic solution and pentoxifylline were not used (p<0.05).

We found that the group in which penoxifillin was used together was less affected by sepsis. In our study, an increase in PMN values was detected in all groups with sepsis. When the groups were compared with each other concerning PMN values, there was a significant difference between the PMN values of the PX group and the other three groups (p<0.05). Between the group using the hypertonic solution and the group using pentoxifylline, no significant difference was found (p>0.05), but when hypertonic solution and pentoxifylline were combined, a significant difference was found depending on the group in which it did not use hypertonic solution and pentoxifylline (p<0.05).

In our study, high fever was detected in all groups with sepsis. When the fever values of the groups were compared with each other, in the control group, there was a significant difference between the fever values and the other three groups (p<0.05). There was no significant difference between the group using the hypertonic solution and the group using pentoxifylline.

Leukocytosis was also detected in all groups with sepsis. When compared to the leukocyte values, the control group's results had a significant difference from the values of the other three groups (p<0.05). There was a significant difference between the solution group and the pentoxifylline group (p<0.05), and there was a significant difference in the group in which hypertonic solution and pentoxifylline were used together.

Table 1. Comparison s of the groups

	Group I	Group II	Group III	Group IV	p-values
WBC	18281±377,81	15300±373,21	15175±205,93	11327±600,58	P<0.05**
PLT	199400±32960	162700±29079	146000±30570	120625±22670	P<0.05**
PMN	3449±,00065	2584±,010236	33725±,04377	2556±,000833	P<0.05**
Fever	37.9±,07998	37.77±,14237	37.56±15462	37.38±, 14131	P<0.05**

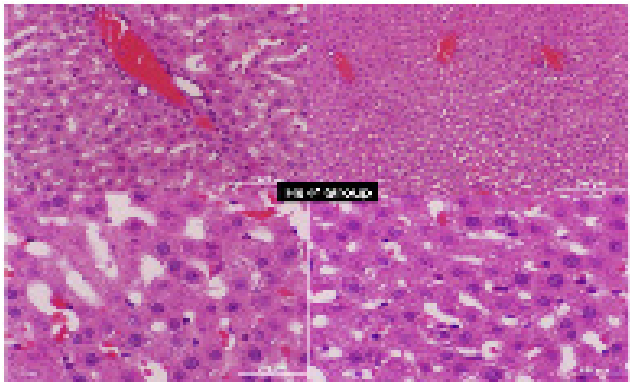

Figure 1. In the sepsis -hypertonic solution+pentoxifylline group, degenerative changes in the liver tissue and no inflammatory cell infiltration were observed in rats (H-E X 20,40).

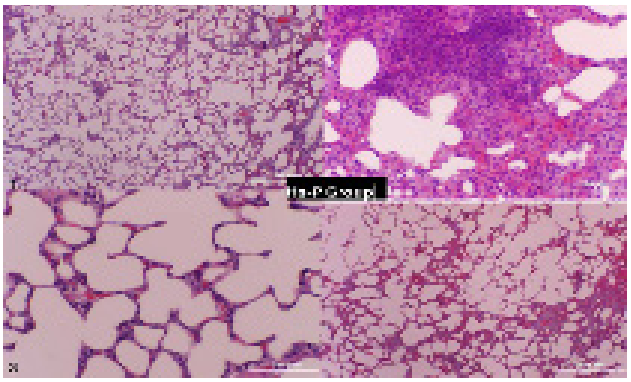

Figure 2. Smooth alveolar structure and minimally limited inflammatory cell infiltration in some interstitial distances in lung tissues in Sepsis HS+PTX Group and rats (H-E X 20,40).

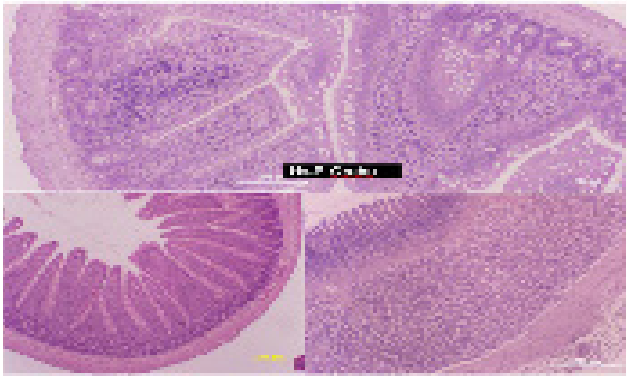

Figure 3. Minimal inflammatory cell increase in villi in the ileum and minimal lymphocyte exocytosis in surface epithelium in rats in Sepsis-HS+PTX Group (H-E X 20,40).

When histopathological findings of liver, ileum, lung were compared, there were no difference between the HS Group and PTX Group (p>0.05). It was determined that there was a significant difference between the control Group (C) and HS+PTX Group (p<0,05).

Discussion

A therapeutic strategy involving the co-administration of HS and PTX was used to successfully treat abdominal sepsis. In the last 20 years, the number of cases of hypovolemia, shock and early sepsis has been limited. The use of hypertonic solutions (4ml/kg b.w) as a first-line is an effective and valid method.

Intestinal obstruction generates an intense inflammatory process that leads to an imbalance in the secretion of fluids, culminating in the loss of fluids and alterations in electrolytes [11]. As assessed by other researchers, bacterial translocation is also a consequence of intestinal obstruction and may be the cause of sepsis in this condition [12-14]. Moreover, intestinal involvement in sepsis involves the release of inflammatory factors that promote local and systemic lesions. Over the 24-hour period of intestinal obstruction and ischemia in this model, there was an increased inflammatory response and hemodynamic changes that implied a decrease in renal perfusion pressure. The basis of this therapy is the osmotic flow of the endogenous fluid [15]. It is based on the transition from the ıntracelluler space according to the gradient in early sepsis. Early sepsis. In the periods of post-traumatic hemorrage and shock adenosine increases. It increases due to loss of triphosphate (ATP) and because the cell membrane cannot change fluid accumulation occurs in the cells. Thus, the fluid from the intracellular space mobilization has two important advantages. First, the plasma volume increases 3–4 times; secondly, bringing fluid exchange in the endothelial cell membrane closer to its normal level increases microcirculatory blood flow.

This model represents a strangulation and perforation of small bowel obstruction, acute abdominal sepsis, a common problem in the emergency department. Studies that have evaluated HS and PTX combinations generally have not assessed the effects of each treatment alone, as we did in this study. Faveng et al. Demonstrated that fluid resuscitation improves intestinal blood flow and reduces the mucosal damage associted with strangulation obstruction. In some situations, the use of a large amount of fluids causes intraabdominal hypertension [16].

We included leukocyte and thrombocyte counts that were examined in these patients. The percentage of leukocyte count was high in both groups, but no statistical difference could be detected between them. The platelet count was statistically significantly lower in the bacteremic group.

An experimental study by Coimbra et al. Showed that hypertonic solution and pentoxifylline decrease the number of leukocytes and PMN in rats with hemorrhagic shock and sepsis. In an experimental study by Coimbra et al. [17], İn rats with sepsis, pentoxifylline significantly reduced the polymorphonuclear leukocyte count and reduced tissue damage.

Zenaide et al.'s experimental study in dogs with septic shock found that pentoxifylline had antipyretic properties and decreased leukocyte values [18].

Rohan et al. in their experimental study found that pentoxifylline provides neutrophil activation [19].

In an experimental study by Shi et al. hypertonic solutions were used in rats with a trauma-hemorrhagic shock-sepsis model. They found that neutrophil values decreased compared to the control group [20].

Coimbra et al. in an experimental study carried out with shock and sepsis criteria, administered hypertonic solutions and pentoxifylline in separate control groups in patients and in the experimental group, both were used together. In the group where solution and pentoxifylline were used together, compared to the other groups, revealed that polymorphonuclear neutrophil values decreased more [21].

In a study by Jessica et al., findings revealed that pentoxifylline reduced neutrophil activation with decreases via CD35 and CD66 on the cell surface [22]. Homma et al conducted a study and they examined the histological changes in the liver of rats. Sepsis created dense polymorphonuclear cells in the liver of animals compared to the control group infiltration and mitochondrial disruption were observed [23].

Flamand et al. Performed cecal-ligation and perforation in their experimental study. In the sepsis model created using pentoxifylline alone, tissue damage (liver, small intestine, lung) has not been shown to inhibit [24].

In our study, histopathological examination of the liver was observed in the group with sepsis. Up to balloon degeneration cloudy and hydropic swelling-degenerative changes were seen. Single-cell necrosis, in a single or a few foci, in various localizations, focal foci of necrosis were observed. Small amounts of hemosiderin accumulation in Kupfer cells or hepatocytes were seen. In minimal to moderate mononuclear sinusoids, there were inflammatory cells consisting of polymorphonuclear, eosinophilic leukocytes. More focal or diffuse microvesicular appearance in hepatocytes was seen in affected rats. There was degeneration. In some rats, abscess was observed in the liver. In our study, liver damage was more prominent in the control group than in others.

There was a significant difference between the three groups ($p<0.05$). The hypertonic solution was used.

There was no significant difference between the pentoxifylline group and the pentoxifylline group ($p>0.05$).

There was asignificant difference between hypertonic solution -pentoxyfilline and other groups. In the group in which hypertonic solution and pentoxifylline are used together we found that it was less affected by sepsis in terms of liver damage.

The lungs initiate the event in the pathogenesis of lung injury cytokines released from macrophages. Cytokines are mediated by neutrophils and mononuclear cells, providing migration. Neutrophils increase damage by releasing free oxygen radicals and proteases. Meanwhile, the coagulation system is activated in the pulmonary capillaries leading to the formation of microthrombi. Pro-inflamation resulting from trombosis depending on the inflamatorymolecules and endothelial damage.This process damages vasculer structure and increases vasculer permeability.

As a result, first, the interstitium and then the alveoli are filled with protein-rich edema fluid. Edema causes surfactant dysfunction and microatelectasis. At the end of its progression, pulmonary fibrosis may develop [25].

Jessica et al.'s study determined that pentoxifylline reduces the destructive effect of neutrophils on the lungs and decreases proinflammatory synthesis, and effects with CD35 and CD66B cytokines [22].

In an experimental study conducted by Shi et al [20], hypertonic solutions have positive effects on lung permeability in rats that developed sepsis and trauma-schock. Histologically, it was observed that the lungs were less affected than the control group. It is thought that they affect by reducing neutrophil degranulation.

Coimbra et al. [21], in an experimental study using hypertonic solutions on neutrophil activation and tumor necrosis factor synthesis, hypertonic solutions, showed that pentoxifylline had less effect than its concomitant use.

In our study, we provided rats with sepsis and used separately hypertonic solutions and pentoxifylline. We found histopathologically septic congestion in the lungs, interstitial and intra-alveolar edema, hemorrhage, hemosiderin deposition, bronchial walls inflammatory cell communities consisting of lymphocytes-histocytes, a fibrinoid substance in the vessels accumulation and polymorphonuclear infiltration. There was no significant difference between hypertonic solutions, pentoxifylline, control group ($p>0.05$). However, it was affected more than the other groups when it was used together and a significant difference was found ($p<0.05$). In Shi et al.'s study, they formed a hemorrhagic sepsis-trauma in rats. In this shock model, they found that hypertonic solutions were more protective for intestinal structure than Ringer's lactate solution [20]. In histological examination, submucosal edema and villus necrosis were less common in rats when hypertonic solutions are used than Ringer's lactate solution used in rats.

Flamand et al. Provided cecal ligation-perforation in rats with an experimental study on their results. Pentoxifylline protected microvascular circulation in rats with sepsis. However, according to the control group, no meaningful effect was observed in the rats with sepsis and also no significant difference was found between the histopathological examinations [24].

In our study, in the histopathological examination of the ileum, inflammatory cell increase and flattening, hyperplasia and reactive changes in lymphoid tissue were observed. When all groups were compared among themselves, the histopathological findings observed in the ileum showed a statistically significant difference regarding changes ($p<0.05$).

Conclusion

The findings obtained in this study showed that hypertonic solutions and pentoxifylline together with the therapeutic properties and benefits of its use in the early stages of sepsis, hematologically, had a statistically significant difference in clinical and histopathological terms. Using hypertonic solutions and pentoxifylline together prevents the progression of sepsis in the early stages of sepsis. These drugs can add treatment of sepsis with clinical studies.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

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Conflict of interest

None of the authors received any type of financial support that could be considered potential conflict of interest regarding the manuscript or its submission.

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